

## Minimal persistent inflammation is present at mucosal level in patients with asymptomatic rhinitis and mite allergy

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*The natural exposure to house dust mites causes sensitization in genetically susceptible patients. Persistent exposure of sensitized patients causes chronic inflammation, and consequently, hyperreactivity, thus promoting the development of clinical features. Recently, intercellular adhesion molecule-1 (ICAM-1)/CD54 expression on epithelial cells triggered by allergen has been demonstrated and related to the inflammation caused by the allergic reaction. Therefore we evaluated the possible presence of inflammation (i.e., inflammatory cell infiltrate and ICAM-1/CD54 expression on epithelium) at conjunctival and nasal levels in patients with asymptomatic allergic rhinitis caused by mites, in their relatives living in the same environment, and in healthy volunteers. In addition, the possible relationship between inflammation and house dust mite allergen exposure was evaluated. Conjunctival and nasal scrapings of allergic subjects enrolled in the study showed many inflammatory cells. A mild ICAM-1/CD54 expression on conjunctival and nasal epithelium was detectable in allergic subjects, whereas relatives and healthy volunteers showed few inflammatory cells and no ICAM-1/CD54 expression on epithelial cells. A detectable level of house dust mite, sufficient to cause sensitization, was found in all houses. This study demonstrates a minimal persistent inflammation at conjunctival and nasal levels constantly detectable in patients without symptoms who are sensitized to mites and continuously exposed to the natural allergens. (J ALLERGY CLIN IMMUNOL 1995;96:971-9.)*

**Key words:** Dermatophagoides pteronyssinus, D. farinae, house dust mites, exposure level, minimal persistent inflammation, hyperreactivity, inflammatory infiltrate, ICAM-1/CD54

Epidemiologic surveys and studies of dust mite avoidance support a role for house dust mites in the pathogenesis of allergic disease.<sup>1,2</sup> Three species (i.e. *Dermatophagoides pteronyssinus*, *D. farinae*, and *D. microcerot*) are recognized as important sources of allergen in house dust<sup>3,4</sup> and among them, three

### Abbreviations used

ICAM-1: Intercellular adhesion molecule-1  
mAb: Monoclonal antibody

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main groups of allergens, namely the Der 1, Der 2, and the Der 3 groups, have been purified.<sup>5-7</sup> Recently, species-specific assays able to detect the mite levels (Der p 1, Der f 1, and Der 2) in houses of allergic patients have become available.<sup>8,9</sup>

Moreover, concerning house dust mite exposure levels, the comparison between mite-sensitive patients with acute relapse and patients who are free of symptoms has led to the proposal of reference levels of dust mite allergens for both sensitization and symptom appearance in allergic patients. In this regard, Platts-Mills et al.<sup>10,11</sup> established the

level of 2  $\mu\text{g}$  of the Group 1 mite allergens (i.e., Der p 1 plus Der f 1) per gram of dust (equivalent to 100 mites per gram) as a risk factor for sensitization and the development of allergic asthma and the level of 10  $\mu\text{g}/\text{gm}$  of dust as a major risk factor for the development of acute asthma in patients allergic to mites.

On the other hand, allergic asthma is presently considered a chronic inflammatory process with intermittent acute relapses.<sup>12-14</sup> The allergic inflammation is characterized by loss of surface-lining epithelium and epithelium shedding and by thickening of the reticular layer beneath the basal lamina of the epithelium until subepithelial fibrosis, mucus hypersecretion, changes in the microvasculature, and a dense inflammatory cell infiltrate have been detected.<sup>12-14</sup> Epithelial damage and shedding are mainly caused by eosinophil infiltration, because cationic eosinophilic proteins with neutrophil oxygen metabolite products exert toxic effects on airway epithelium.<sup>12-15</sup> Eosinophil adhesion to epithelial cells is regulated by adhesion molecules.<sup>16-19</sup> In particular, eosinophil surface expresses leukocyte functional antigen-1,<sup>16, 17</sup> an integrin for which the natural ligand is intercellular adhesion molecule-1 (ICAM-1)/CD54.<sup>20</sup> In this regard, we have recently demonstrated that conjunctival and nasal epithelial cells are induced to express ICAM-1/CD54 both during the acute phase of an allergic reaction and after allergen-specific challenge.<sup>21, 22</sup>

Furthermore, a *minimal persistent inflammation* has been demonstrated in asthmatic patients during clinical latency.<sup>13</sup> All of these concepts are very important to the development of a more adequate treatment for patients with allergy in order to avoid possible irreversible damage.<sup>23-25</sup>

On the basis of this evidence, the aim of this study was to evaluate the possible presence of inflammation (i.e., inflammatory cell infiltrate and adhesion molecule expression) at conjunctival and nasal levels in patients with asymptomatic allergic rhinitis caused by mites and the possible relationship to house dust mite concentration.

## METHODS

Two different groups were selected for this study. Group 1 underwent conjunctival evaluation, and group 2 underwent nasal assessment. All subjects gave informed consent, and the trial was approved by the department ethical committee.

### Group 1

Twenty patients (8 men and 12 women, aged 18 to 46 years) with allergic rhinitis caused by *D. pteronyssinus* and *D. farinae* were enrolled in the study. The diagnosis

of allergic rhinitis was based on history of 2 or more years of classical signs and symptoms of perennial rhinitis, positive skin prick test and RAST results for mites (*D. pteronyssinus* and *D. farinae*). No patient had positive skin test or RAST responses to any other allergen, and no patient had symptoms at any other time. No sign or symptom of conjunctivitis was evident for at least 3 weeks, and results of local examination were negative at the time of admission.

Twenty healthy volunteers (5 men and 15 women, aged 19 to 65 years) were enrolled as a control group: they were first-degree relatives (parents or siblings) of the patients (one for each patient), living in the same houses as the patients. All relatives had negative history of allergic disease and negative skin prick test and RAST results for a common panel of allergens.

### Group 2

Twenty subjects (9 women and 11 men, aged 18 to 53 years) were studied. Ten of them were healthy volunteers with negative history of allergy and negative skin prick test and RAST results for a common panel of allergens. Ten had perennial rhinitis caused by *D. pteronyssinus* and/or *D. farinae* for at least 2 previous years. All patients with allergy had positive skin prick test and RAST results for house dust mites (*D. pteronyssinus* and *D. farinae*). No patient had positive skin test or RAST responses to any other allergen, and no patient had symptoms at any other time. All patients had been free of symptoms for at least 3 weeks (i.e., they did not have nasal obstruction, rhinorrhea, itching, and sneezing for at least 3 weeks). On admission, the local examination did not reveal any sign of rhinitis.

All subjects included in the study had no ocular diseases, they did not wear contact lenses, they did not have any upper or lower respiratory airway disease, they were nonsmokers, and they had not taken any topical or systemic drugs for 1 month before entering the study, and they had not received specific immunotherapy.

### Dust samples

Dust from bedroom floors was collected with a vacuum cleaner, fitted with a filter holder. All dust samples were collected during January. The dust was mixed and shaken with glass beads (diameter = 5 mm), then sieved through a 0.3 mm mesh screen to obtain fine dust. The dust was extracted at 15% (wt/vol) with phosphate buffer (0.1 mol/L, pH 7.4). After centrifugation (12,000 rpm for 5 minutes), the supernatant was used as a dust sample.<sup>7, 9</sup>

### Quantitation of mite allergens

The mite allergens (i.e., Der p 1 and Der f 1) were measured by a solid-phase ELISA method; allergens belonging to Group II (i.e., Der p 2 and Der f 2) were measured together by a commercially available radioimmunoassay method. (Abelló, Madrid S.A., Spain). Briefly, a determined amount of the samples prepared as described above is added to a microwell sensitized with a monoclonal antibody (mAb) specific to Der p 1 or Der f 1 after incubation and washing, a determined amount of

**TABLE I.** Mite allergen exposure of allergic subjects and their relatives who underwent conjunctival scraping

House	Der p 1	Der f 1	Der 1*	Der 2†	Der 1:Der 2‡	Exposure level§
1	0.06	1.06	1.12	2.8	0.4	Low
2	0.2	18	18.2	15.5	1.17	Very high
3	1.8	1	2.8	2.27	1.23	High
4	0.2	3	3.2	0.7	4.57	High
5	1.67	20.6	22.27	14.34	1.55	Very high
6	0.06	3.3	3.36	0.8	4.2	High
7	<0.05	10	10	4.06	2.46	High
8	<0.05	4	4	0.2	20	High
9	<0.05	1.5	1.5	1.5	1	Low
10	<0.05	2	2	0.2	10	High
11	0.34	11	11.34	6.9	1.64	Very high
12	0.16	1.5	1.66	1.2	1.38	Low
13	2.62	5.6	8.22	6.8	1.21	High
14	0.36	2.8	3.16	1.33	2.37	High
15	0.08	0.6	0.68	0.87	0.78	Low
16	0.4	2.8	3.2	1.47	2.17	High
17	0.25	6.8	7.05	1.4	5.0	High
18	0.18	1.09	1.27	1.6	0.79	Low
19	<0.05	0.72	0.72	0.53	0.74	Low
20	<0.05	34	34	8	4.25	Very high

Allergen levels are expressed as micrograms per gram of fine dust.

\*Der p 1 and Der f 1 add up to Der 1.

†Der p 2 and Der f 2 add up to Der 2.

‡Ratio of Der 1 to Der 2.

§According to Platts-Mills et al.<sup>10,11</sup>

a pool of human sera belonging to mite-sensitive patients is added. After washing, an mAb specific for human IgE, coupled with galactosidase is added; finally, the proper substrate is added and the color is read at 405 nm.<sup>26</sup> For Group II allergens, the procedure is similar, but the allergen-specific mAb recognizes Der p 2, as well as Der f 2; the mAb specific for human IgE is labeled with iodine 125, and the radioactivity bound is determined in a gamma counter.<sup>6</sup> Allergen concentration in the samples is then calculated through proper standard curves.

### Cytologic assessment

According to our previous reports,<sup>21, 22, 27, 28</sup> conjunctival scrapings for cytologic assessment were obtained from both eyes of allergic subjects and their relatives. After topical anesthesia (ossibuprocaine 4 mg/ml, 1 drop in each eye) was induced, the upper tarsal conjunctiva was scraped with a sterile Kimura spatula. Specimens were immediately spread on glass slides and air-dried. Specimens were stained with May-Grünwald-Giemsa dye and read by microscope (Leitz Laborlux Microscope [Wild Microscopes, Rockleigh, N.J.], X500 focus). The number of inflammatory cells (neutrophils, eosinophils, lymphocytes, and monocytes) was considered as a total number of any cell type per microscopic field; the data are expressed as a mean of 10 fields. Slides were

examined by two investigators masked to the identity of the samples.

Nasal scrapings were performed with a cotton-tipped swab (Minitip Coulrette; Becton-Dickinson, Milan, Italy) applied to both nostrils after nasal lavage with Ringer's solution (10 ml). Specimens were obtained from the middle third of the inferior turbinate. After scraping, the swab was immersed in phosphate-buffered saline solution (2 ml) to allow cell elution. The suspension was filtered and cytospun; slides were prepared with standard technique; examination of samples was identical to that for the conjunctival samples. In addition, toluidine blue stain (pH = 2.5) was used to identify metachromatic cells.<sup>29</sup>

A sensitive immunoenzymatic alkaline phosphatase-monoclonal anti-alkaline phosphatase complex procedure modified from the method of Cordell et al.<sup>30</sup> was used. Specimens were air-dried at room temperature for 30 minutes and submitted to 1:50 dilution of purified ICAM-1/CD54 mAb (1 mg/ml, 84H10, IgG<sub>1</sub>; Immunotech, Marseille, France)<sup>31</sup> or to 1:100 dilution of anti-cytokeratin mAb (aCK19, IgG<sub>1</sub>; DAKO, Milan, Italy) as a marker of epithelial cells. After washing in phosphate-buffered saline pH 7.6, samples were incubated with rabbit anti-mouse immunoglobulin, followed by alkaline phosphatase-monoclonal anti-alkaline phosphatase complex. Afterwards, specimens were incubated in sub-

**TABLE II.** Skin prick test and serology results for allergic patients who underwent conjunctival scraping

House	Allergic patients			
	Skin prick test		RAST	
	Der p	Der f	Der p	Der f
1	+++	++++	II	III
2	++++	++++	IV	IV
3	+++	++++	IV	IV
4	++++	++++	IV	IV
5	+++	+++	III	IV
6	+++	++++	III	IV
7	++	+++	II	III
8	++++	++++	III	IV
9	+++	+++	III	IV
10	+++	++++	III	IV
11	+++	++++	IV	IV
12	++	+++	II	III
13	++++	++++	IV	IV
14	++++	++++	IV	IV
15	++++	++++	IV	IV
16	+++	+++	III	III
17	++	+++	II	III
18	++++	++++	IV	IV
19	++	+++	II	III
20	+++	++++	III	IV

The patients' relatives had negative skin prick test and serology results for a panel of common allergens, including house dust mite.

**TABLE III.** Skin prick test and serology results for subjects who underwent nasal scraping

Allergic patient	Skin prick test		RAST	
	Der p	Der f	Der p	Der f
1	+++	++++	III	IV
2	+++	+++	III	III
3	+++	+++	IV	IV
4	++++	++++	IV	IV
5	+++	+++	III	III
6	+++	++++	III	III
7	++++	++++	IV	IV
8	+++	+++	III	III
9	+++	++++	III	IV
10	++	+++	III	II

Healthy volunteers had negative skin prick test and serology results for a panel of common allergens, including house dust mite.

strate solution containing basic new fuchsin, naphthol. As biphosphate, and levamisole as an inhibitor of endogenous alkaline phosphatase (Sigma, St. Louis, Mo.). In control samples either mAb or anti-mouse immunoglob-

**TABLE IV.** Cytologic data of allergic patients and their relatives who underwent conjunctival scraping

House	Allergic patients				Relatives					
	TC	N	L	E	CD54	TC	N	L	E	CD54
1	7	7	0	0	1	2	2	0	0	0
2	9	5	2	2	2	1	1	0	0	0
3	10	8	1	1	1	2	2	0	0	0
4	13	5	4	4	2	3	2	1	0	0
5	8	5	2	1	1	0	0	0	0	0
6	11	11	0	0	1	3	1	2	0	0
7	8	8	0	0	1	2	2	0	0	0
8	9	7	1	0	1	2	1	1	0	0
9	7	5	2	0	1	2	2	0	0	0
10	8	5	2	1	1	3	3	0	0	0
11	9	3	6	0	2	0	0	0	0	0
12	8	6	2	0	1	0	0	0	0	0
13	7	6	1	0	1	3	2	1	0	0
14	13	4	4	5	2	0	0	0	0	0
15	10	2	5	3	1	3	3	0	0	0
16	8	6	2	0	1	0	0	0	0	0
17	7	5	2	0	1	0	0	0	0	0
18	12	4	3	5	1	2	0	2	0	0
19	6	3	3	0	1	0	0	0	0	0
20	9	7	2	0	1	0	0	0	0	0

TC, Total number of inflammatory cells; N, neutrophil; L, lymphocyte; E, eosinophil; CD54, expression on conjunctival epithelium.

Comparison between allergic subjects and their relatives was performed by Mann-Whitney U two-tailed test: TC,  $p < 0.001$ ; N,  $p < 0.001$ ; L,  $p < 0.001$ ; E,  $p < 0.001$ ; CD54,  $p < 0.001$ .

ulin was omitted. As a negative isotype control for ICAM-1/CD54 staining on epithelial cells, an anti-T-lymphocyte (CD3) mAb (OKT3, IgG<sub>1</sub>; Ortho Diagnostic, Raritan, NJ.) at 1:20 dilution of the stock solution (provided by the manufacturer) was used. The dilutions were established on the basis of previous titration experiments. All preparations were counterstained with Carazzi's hematoxylin and examined by two investigators blinded to the identity of the samples.

ICAM-1/CD54 expression on epithelial cells was scored according to our previous reports by a 5-point rating scale from 0 to 4, where 0 = no positive cells; 1 = mild positivity on 25% of epithelial cells; 2 = mild positivity on 75% of epithelial cells; 3 = intense positivity on 75% of epithelial cells; and 4 = very intense positivity on all epithelial cells.<sup>21, 22</sup>

#### Statistical analysis

Statistical analysis of results was performed by the Mann-Whitney U test to determine differences between groups. Spearman's test was used to evaluate the possible correlation between house dust mite exposure and cytologic data of allergic patients. A probability value of 0.05 or less was considered statistically significant.

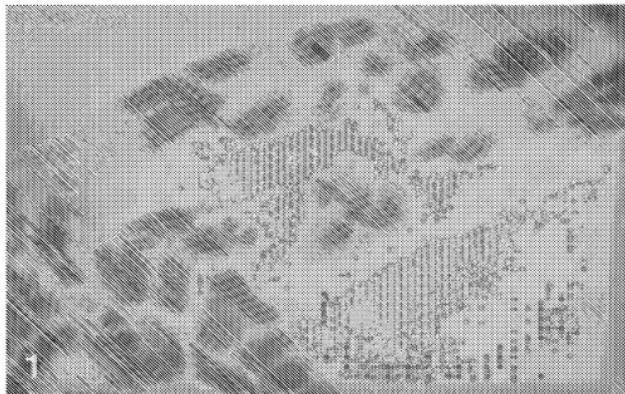


FIG. 1. Inflammatory cell infiltrate represented by several neutrophils and ICAM-1/CD54 expression on conjunctival epithelium (red staining) in a mite-sensitized patient without symptoms.

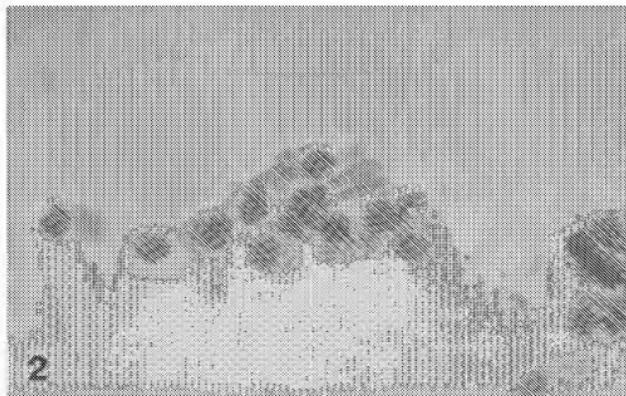


FIG. 2. Conjunctival epithelium in relative of mite-sensitized patient. Neither ICAM-1/CD54 expression on epithelium nor inflammatory cells can be seen.

## RESULTS

### Dust samples

The dust allergen contents of 20 houses of patients with allergy to *Dermatophagoides* species are reported in Table I. The allergen levels of Der 1 and Der 2 in the dust ranged, respectively, from

0.68 to 22.27 and from 0.2 to 15.5  $\mu\text{g}/\text{gm}$  fine dust. Comparing the amounts of Der p 1 and Der f 1, we reported that *D. farinae* was dominant in these houses. With regard to the exposure level, according to the literature,<sup>10, 11</sup> a low level was present in six houses, high in 10 and very high in four.



FIG. 3. ICAM-1/CD54 expression on nasal epithelium (red staining) in a mite-sensitized patient without symptoms.

TABLE V. Cytologic data of allergic patients and healthy volunteers who underwent nasal scraping

TC	Allergic patients				Healthy volunteers			
	N	E	M	CD54	TC	N	E	M
1	41	40	1	0	1	2	2	0
2	34	25	5	4	2	1	0	1
3	52	40	12	0	2	0	0	0
4	59	45	14	0	2	1	1	0
5	14	12	1	1	1	0	0	0
6	9	4	5	0	1	1	1	0
7	15	10	5	0	2	2	2	0
8	6	4	1	1	2	1	0	1
9	2	2	0	0	1	1	1	0
10	5	3	2	0	2	1	1	0

TC, Total number inflammatory cells; N, neutrophil; E, eosinophil; M, metachromatic cells; CD54, expression on nasal epithelium.

Comparison between allergic subjects and healthy volunteers ( $n = 10$ ) was performed by Mann-Whitney U two-tailed tests:

TC,  $p < 0.01$ ; E,  $p < 0.001$ ; N,  $p < 0.001$ ; M, not significant; CD54,  $p < 0.001$ .

#### Skin prick tests and serology

Table II shows the skin prick test and RAST values of allergic patients and their relatives who underwent conjunctival evaluation. Table III shows the skin prick test and RAST values of subjects who underwent nasal scrapings. All aller-

gic patients had positive skin prick test results (ranging from ++ to +++) and positive RAST results (ranging from class II to IV). All relatives and normal control subjects had negative results for both skin prick tests and RAST. The predominant positivity to *D. fariniae* (skin prick test and RAST) demonstrated in "group 1" subjects with allergy fit very well with Der f 1 allergen levels in their houses.

#### Cytologic evaluation

Table IV shows total and single cell numbers and ICAM-1/CD54 expression on conjunctival epithelial cells in allergic patients. All patients showed a high number of inflammatory cells at the conjunctival level (mainly neutrophils), ranging from 6 to 13 (Fig. 1). A mild positive expression of ICAM-1/CD54 on conjunctival epithelial cells was present in all allergic patients (positivity scores of 1 or 2) (Fig. 1).

Relatives showed few inflammatory cells (total number ranging from 0 to 3, mainly neutrophils); and in particular, no eosinophil was detected in any relative. No expression of ICAM-1/CD54 on conjunctival epithelial cells was present in relatives (Fig. 2). The statistical analysis demonstrated a significant difference for cellular parameters (i.e., inflammatory cell number and ICAM-1/CD54 expression) between allergic patients and their relatives ( $p < 0.001$ ). In addition, concerning allergen

exposure, Der 1 concentration above the reference limits<sup>41</sup> was detectable, although no correlation was detected when cellular data (including ICAM-1/CD54 expression) were compared with mite levels.

Table V shows total and single cell numbers and ICAM-1/CD54 expression on nasal epithelium of allergic patients and healthy volunteers. All allergic patients showed a wide infiltration of inflammatory cells in the nasal mucosa (mainly neutrophils), ranging from 2 to 59. In three of them we observed the presence of metachromatic cells; in addition, a mild expression of ICAM-1/CD54 molecule was present on nasal epithelial cells (Fig. 3). Few inflammatory cells (total number ranging from 0 to 2, mainly neutrophils) were detected in healthy volunteers, and no ICAM-1/CD54 expression was evident on epithelial cells. Significant difference for each cellular parameter (i.e., inflammatory cell number and ICAM-1/CD54 expression) appeared between the two studied groups ( $p < 0.001$ ), that is, between allergic subjects and healthy volunteers.

Furthermore, we were able to re-evaluate some allergic patients included in groups 1 and 2 when they had symptoms. As expected, a higher number of inflammatory cells and a more marked CD54 positivity on epithelium were demonstrated at both conjunctival and nasal levels (data not shown).

## DISCUSSION

House dust is a complex mixture of organic and inorganic substances, and a large number of several biologic sources have been demonstrated to be able to sensitize predisposed subjects.<sup>1,2</sup> Particularly, mites (mainly *D. farinae* and *D. pteronyssinus*) are the most important cause of sensitization. Clinical study (e.g., bronchoalveolar lavage and biopsies)<sup>12-15,24</sup> and experimental challenge (e.g., allergen-specific provocation tests) have demonstrated that the allergic reaction is an inflammatory process<sup>20,21,32,33</sup>; particularly, it is now widely accepted that both allergic asthma and allergic rhinitis are chronic inflammatory diseases characterized by infiltration of the airways by inflammatory cells (e.g., lymphocytes, macrophages, neutrophils, and eosinophils), edema of the bronchial mucosa, thickening of the basal membrane, and disruption and desquamation of respiratory epithelium.<sup>12,13</sup> In this regard, a mild degree of inflammation at the bronchial level has been reported even in asthmatic patients during clinical remission, namely minimal persistent inflammation.<sup>13</sup> Similarly, patients with perennial rhinitis demonstrated a sig-

nificant number of eosinophils and increase of inflammatory mediators in nasal secretions, as well as inflammatory cell infiltrate and mucosal damage in nasal biopsy specimens.<sup>29,33</sup>

For this reason, studies regarding the natural exposure to allergens may clarify these reports. The possible role played by mite allergens in the pathogenesis of the allergic reaction can be divided into three phases: (1) the initial sensitization, (2) the development of hyperreactivity, and (3) the triggering of acute relapses.<sup>12-14</sup> Thus the allergen exposure causes sensitization in genetically susceptible patients, the continuous exposure even at a moderate level can trigger and sustain local inflammation with epithelial damage (i.e., caused by eosinophil infiltrate) and consequently hyperreactivity, thus increasing the risk of developing clinical manifestations.<sup>34</sup> For these reasons, at the first and the second international workshops on dust mite allergy, a level of 2  $\mu$ g of these Group I mite allergens (i.e., Der p 2 plus Der p 1) per gram of house dust mite was proposed as a risk factor for sensitization and development of allergic symptoms.<sup>10,11</sup>

Along these lines, these data represent the first evidence of a conjunctival and nasal inflammation (i.e., inflammatory cellular infiltrate, mainly neutrophils, and ICAM-1/CD54-positive expression on epithelial cells) in patients with allergic rhinitis and sensitization to house dust mite evidenced during clinical latency (i.e., during a symptom-free period). It should be emphasized that the dust mite evaluation revealed that all houses of allergic patients contained a mite level sufficient to cause sensitization and, consequently, inflammation. In addition, the inflammatory process appeared to be restricted to allergic patients, whereas relatives and healthy volunteers did not show a significant inflammatory cell infiltration, although they were living in the same conditions. In particular, no eosinophil was detected in relatives or healthy volunteers. Although a significant correlation between house dust mite allergen exposure level and inflammatory parameters (i.e., cellular and immunologic data) is absent, these data suggest that even a low exposure level (in any case  $> 2 \mu\text{g}/\text{gm}$  dust) is sufficient to induce a subclinical inflammation. In our region it is very rare to find houses with no detectable levels of dust mite allergens because the mediterranean climate is favorable for mite growth.

As suggested by Platts-Mills<sup>10,11</sup> long-term and low-level exposure can trigger an inflammatory response and, subsequently, an increase in hyperreactivity.<sup>34</sup> In this regard, we have recently dem-

onstrated that histamine-induced hyperreactivity at the conjunctival level only in dust mite-sensitive patients compared with pollen-sensitive patients out of the pollen season.<sup>35</sup> Those data strongly support the role of a persistent allergen exposure and a possible relationship between allergic inflammation and hyperresponsiveness. Furthermore, the persistent inflammatory infiltrate and the changes in surface adhesion molecule pattern expressed by inflamed epithelium of different target organs of allergic reactions, specifically the expression of ICAM-1/CD54,<sup>36,37</sup> are also relevant as far as the susceptibility to viral infections is concerned. About 90% of rhinoviruses used ICAM-1/CD54 as a receptor, their binding site being distinct from, although overlapping with leukocyte functional antigen-1.<sup>38</sup> Therefore a possible causal link can be hypothesized between exacerbation of asthma and viruses, as recently reviewed in several epidemiologic studies.<sup>39</sup> At this stage it might be hypothesized that the demonstrated *minimal persistent inflammation* is also capable of inducing a discrete ICAM-1/CD54 expression on respiratory epithelium, which may become susceptible to virus binding.

This study demonstrates that at both conjunctival and nasal levels, a *minimal persistent inflammation* is constantly detectable in patients with sensitization to mites who are free of symptoms, and a detectable level of dust mite allergen was found in all houses of studied subjects. This experimental evidence also points out that in other situations of persistent and discrete exposure to allergens other than mites, a *minimal persistent inflammation* might be detected even during clinical latency. These concepts are underlined by the assumption that allergic asthma is a chronic inflammatory disease, characterized by a deep and continuous remodeling of the primary anatomic structure of the target organ (i.e., subepithelial fibrosis). Therefore fundamental suggestions arise from these data concerning the evaluation and therapeutic management of patients with house dust mite allergy. First, ICAM-1/CD54 expression on epithelium might be considered a sensitive marker of inflammation, because its presence is restricted to allergic subjects even during clinical latency; second, house dust mite avoidance measures and continuous antiinflammatory and antiallergic therapy at low and effective doses represent the appropriate strategy for treatment of allergic subjects.

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